Injection volume: 25
Detector: UV 229

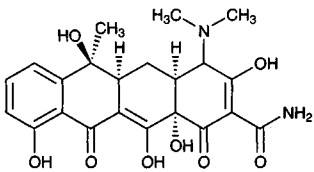
CHROMATOGRAM
Retention time: 10.81 (A), 5.44 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Tetracycline



Molecular formula: $C_{22}H_{24}N_2O_8$
Molecular weight: 444.44
CAS Registry No.: 60-54-8, 6416-04-2 (trihydrate), 64-75-5 (HCl), 1336-20-5 (phosphate)
Merck Index: 9337
Lednicer No.: 1 212

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the

residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 274.8

CHROMATOGRAM
Retention time: 9.888

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: eggs, tissue
Sample preparation: Prepare a metal chelate affinity chromatography (MCAC) column by adding 1.5 mL of thoroughly mixed Chelating Sepharose Fast-Flow suspension in EtOH:water 20:80 (Pharmacia) to a 150 × 10 glass column, allow to drain, wash with three 2 mL portions of water, add 2 mL 10 mM copper(II) sulfate in water, wash with two 2 mL portions of water. Condition an SBD-RPS extraction membrane (3M Company, St. Paul, MN) with 2 mL MeOH and 2 mL 100 mM HCl. Add 20 mL 100 mM pH 4.0 sodium succinate buffer to 3 g pig kidney, pig muscle, cow liver, or whole chicken egg, vortex for 1 min and shake for 10 min on a horizontal shaker. Add 20 mL MeOH, sonicate for 5 min and centrifuge at 2666 g for 10 min at 4°. Filter the supernatant through a Whatman 541 filter paper. Add the clear supernatant to the MCAC column. Wash sequentially with 2 mL 100 mM sodium succinate buffer, 2 mL water, 2 mL MeOH, 2 mL water, and with 500 μL McIlvaine-EDTA-NaCl buffer. Elute with 3 mL McIlvaine-EDTA-NaCl buffer and adjust the eluate to pH 1.3 with 400 μL 4 M HCl. Add the eluate directly to the extraction membrane to prevent crystallization of EDTA. Wash the membrane with 1 mL 100 mM HCl and elute with four 250 μL portions of MeOH:25% ammonia 97:3, evaporate the eluate to dryness under the nitrogen at 40°. Reconstitute the dry residue with 250 μL 10 mM oxalic acid in water, vortex, sonicate. Inject a 100 μL aliquot. (The sodium succinate buffer was 100 mM succinic acid, pH adjusted to 4.0 with 10 M NaOH. Prepare the McIlvaine buffer by dissolving 12.9 g citric acid monohydrate and 10.9 g Na_2HPO_4 in 1 L water. The McIlvaine-EDTA-NaCl buffer was 100 mM EDTA and 500 mM NaCl in McIlvaine buffer. Protect all solutions from light.)

HPLC VARIABLES
Guard column: 5 × 3.0 PLRP-S (Polymer Laboratories)
Column: 250 × 4.6 8 μ PLRP-S (Polymer Laboratories)
Mobile phase: Gradient. A was 10 mM oxalic acid in water adjusted to pH 2.0 with 4 M HCl. B was MeCN. A:B from 85:15 to 60:40 over 16 min.
Flow rate: 1
Injection volume: 100
Detector: F ex 406 em 515 following post-column reaction. The column effluent mixed with reagent pumped at 1 mL/min and the mixture flowed through a 600 μL reaction coil to the detector. (Reagent was 5% zirconyl chloride octahydrate in water stored at 4°.)

CHROMATOGRAM
Retention time: 13.5
Limit of detection: 1.20 ng/g (pig kidney), 0.49 ng/g (pig muscle), 1.01 ng/g (cow liver), 0.23 ng/g (chicken egg)
Limit of quantitation: 3 ng/g (pig kidney)

OTHER SUBSTANCES
Extracted: chlortetracycline, doxycycline, oxytetracycline
Also analyzed: demeclocycline

KEY WORDS
cow; liver; pig; kidney; muscle; chicken; metal chelate affinity chromatography; MCAC; SPE

REFERENCE
Croubels,S.M.; Vanoosthuyze,K.E.I.; Van Peteghem,C.H. Use of metal chelate affinity chromatography and membrane-based ion-exchange as clean-up procedure for trace residue analysis of tetracyclines in animal tissues and egg, *J.Chromatogr.B*, **1997**, *690*, 173–179.

SAMPLE
Matrix: eggs, tissue
Sample preparation: Condition an Anagel-TSK Chelate-SPW column with 25 μL 50 mg/mL copper sulfate in water and 500 μL. Homogenize 2 g sliced chicken liver with 1.2 mL 1 M pH 4 citrate buffer and 12 mL ethyl acetate for 1 min. Homogenize 2 g sliced tissue with 1.2 mL 1 M pH 5 citrate buffer and 12 mL ethyl acetate for 1 min. Shake 2 g blended egg with 1.2 mL 1 M pH 5 citrate buffer and 12 mL ethyl acetate for 15 min. Centrifuge the mixture at 11000 rpm for 10 min, decant the supernatant, reextract the residue with two 12 mL portions of ethyl acetate. Add 10 g anhydrous sodium sulfate to the combined supernatants, swirl, let stand for 5-10 min, filter (Whatman 1PS phase-separating filter paper). Evaporate the filtrate to dryness or to an oily residue on a rotary evaporator under reduced pressure at 40°, reconstitute the residue in 2 mL MeOH by vortexing, filter (0.2 μm syringe filter). Add 1.5 mL of the filtrate to the Anagel column at 0.36 mL/min, wash with 500 μL water, 500 μL MeOH, and 500 μL water. Elute the contents of the Anagel column onto the analytical column with mobile phase A, after 11 min remove the Anagel column from the circuit, elute column B using gradient elution of mobile phase A:B, monitor the effluent from column B. (Prepare 1 M pH 4 or 5 citrate buffer as follows: dissolve 192 g citric acid in approximately 800 mL water, adjust pH value with 1 M NaOH and make up to 1 L with water.)

HPLC VARIABLES
Guard column: 5 × 3 PLRP-S
Column: 150 × 4.6 5 μm Polymer Labs PLRP-S
Mobile phase: Gradient. A:B 100:0 for 11 min, to 0:100 in 10 min, maintain at 0:100 for 10 min. A was buffer. B was MeCN:MeOH:buffer 25:10:65. (Buffer was 100 mM KH_2PO_4 containing 10 mM citric acid, and 10 mM EDTA).
Flow rate: 1
Injection volume: 1500
Detector: UV 350

CHROMATOGRAM
Retention time: 25.2
Limit of detection: 5 ng/g

OTHER SUBSTANCES
Extracted: oxytetracycline, chlortetracycline, demeclocycline

KEY WORDS
chicken; egg; metal chelate affinity chromatography; muscle; liver; salmon; trout; venison; SPE

REFERENCE
Cooper,A.D.; Stubbings,G.W.F.; Kelly,M.; Tarbin,J.A.; Farrington,W.H.H.; Shearer,G. Improved method for the on-line metal chelate affinity chromatography-high-performance liquid chromatographic determination of tetracycline antibiotics in animal products, *J.Chromatogr.A*, **1998**, *812*, 321–326.

SAMPLE

Matrix: milk

Sample preparation: Fill a disposable polypropylene column (Bio-Rad Econo-Pac column) with Chelating Sepharose Fast Flow (Pharmacia) and condition it with 10 mL water, 1.5 mL 100 mM copper sulfate, and 100 mL water. Condition a 6 mL SupelClean ENVI-Chrom P SPE cartridge with 2 mL MeOH and 5 mL water. Homogenize 10 g tissue with 20-30 mL 100 mM pH 4 succinic acid buffer. Centrifuge the homogenate at 2000 g at 10° for 15-20 min. Add the supernatant to the metal chelate affinity column, wash sequentially with 5 mL 500 mM NaCl, 10 mL water, 10 mL MeOH, 10 mL water, and 3 mL McIlvaine buffer, discard the clear effluent. Elute with 8 mL McIlvaine-EDTA-NaCl buffer. Add the eluate to the SPE cartridge under gravity, rinse the column with 2.5 mL water, add the rinse to the SPE cartridge. Wash the SPE cartridge with 2.5 mL water. Dry the SPE cartridge by drawing air through it for 2-3 min. Elute with 5 mL MeOH. Evaporate the eluate to dryness under nitrogen at 40-50°, dissolve the residue in 1 mL water. Inject a 100 μL aliquot. (McIlvaine buffer was 500 mM NaCl and 100 mM EDTA (Carson, M.C. J. AOAC Int. 1993, 76, 329).)

HPLC VARIABLES

Column: 150 × 3.9 5 μm PLRP-S (Polymer Labs, USA)

Mobile phase: MeOH:5 mM oxalic acid 58:42

Flow rate: 0.5

Injection volume: 100

Detector: MS, HP 5989, NICI, high energy dynode, HP 59980B particle beam interface 60°, helium sheath 40-45 p.s.i., source 250°, quadrupole 100°, source pressure 1 Torr with methane reagent gas, m/z 378-483

CHROMATOGRAM

Retention time: 5.12

OTHER SUBSTANCES

Extracted: chlortetracycline, demeclocycline, doxycycline, minocycline, oxytetracycline

KEY WORDS

metal chelate affinity chromatography; cow; SPE

REFERENCE

Carson,M.C.; Ngoh,M.A.; Hadley,S.W. Confirmation of multiple tetracycline residues in milk and oxytetracycline in shrimp by liquid chromatography-particle beam mass spectrometry, *J.Chromatogr.B*, **1998**, *712*, 113–128.

SAMPLE

Matrix: milk, tissue

Sample preparation: Wash column A with 20 mL 5 mg/mL aqueous copper sulfate and column B with 100 mL acetone, 100 mL MeOH, and 100 mL water. Sonicate 10 mL milk or 10 g sliced tissue with 40 mL pH 4.0 succinate buffer for 3 min. (Buffer was 5 g succinic anhydride in 1 L water adjusted to pH 4.0 with 1 M NaOH.) Homogenize for 2 min (Ultra-Turrax), centrifuge at 12000 and 24000 rpm for 5 min, filter the supernatant through a Whatman 541 filter paper. Repeat extraction with 40 mL and 20 mL portions of succinate buffer, load the combined filtrates onto column A. Wash column A with 10 mL water, 30 mL MeOH, and two 10 mL portions of water. Elute tetracycline fraction with 40 mL pH 4.0 succinate buffer containing 100 mM disodium EDTA. After elution wash column A with 10 mL pH 4.0 succinate buffer containing 100 mM disodium EDTA. Load 50 mL combined eluate from column A onto column B, wash with two 100 mL portions of water and elute with 100 mL redistilled MeOH, discard the first 10 mL eluate. Evaporate eluate to a small volume on rotary evaporator at 40° and transfer to pear-shaped flask with three 2 mL portions of redistilled MeOH. Add 100 μL 5% β-mercaptopropionic acid in MeOH, evaporate MeOH under reduced pressure at 40°, reconstitute the residue in 500 μL mobile phase, vortex for 15 s and sonicate for 30 s, inject a 10 μL aliquot. (Column A was a 200 × 20 chelating Sepharose column. Prepare column as follows. Thoroughly mix 5 mL chelating Sepharose suspension (Pharmacia AB), place it in a 200 × 20 glass column, let settle to a 15 mm bed height. Remove liquid excess and load the column by passing 20 mL 5 mg/mL copper sulfate through it. Vortex the column after first 10 mL to remove bubbles, then pass 15 mL pH 4.0 succinate buffer through the column. Wash the column with 20 mL water after use, store in MeOH:water 20:80 at 4°. Column B was a 200 × 20 Amberlite XAD-

2 resin column. Prepare as follows. Wash Amberlite resin sequentially with 100 mL MeOH and 100 mL water, place the resin in a 200 × 2 0 glass column to 100 mm bed height.)

HPLC VARIABLES
Guard column: 10 × 2.1 30-40 μm Lichrosorb RP8
Column: 200 × 3 Lichrosorb RP8
Mobile phase: MeCN:10 mM oxalic acid 50:50
Flow rate: 0.4
Injection volume: 10
Detector: UV 350

CHROMATOGRAM
Retention time: 7.5
Limit of quantitation: 10 ng/g

OTHER SUBSTANCES
Extracted: chlortetracycline, oxytetracycline

KEY WORDS
cow; kidney; milk; sheep; pig; muscle; trout; SPE

REFERENCE
Farrington,W.H.; Tarbin,J.; Bygrave,J.; Shearer,G. Analysis of trace residues of tetracyclines in animal tissues and fluids using metal chelate affinity chromatography/HPLC, *Food Addit.Contam.*, **1991**, *8*, 55–64.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil SAS or 150 × 4.6 5 μm Hypersil SAS
Mobile phase: MeCN:buffer 30:70 (Mobile phase was 340 mL 100 mM citric acid, 5 mL 100 mM trisodium citrate, and 5 mL 100 mM Na_2EDTA made up to 500 mL with MeCN.)
Flow rate: 2
Injection volume: 100
Detector: UV 370

CHROMATOGRAM
Retention time: 3.6

OTHER SUBSTANCES
Simultaneous: furazolidone, oxytetracycline, chlortetracycline

REFERENCE
Murray,J.; McGill,A.S.; Hardy,R. Development of a method for the determination of oxytetracycline in trout, *Food Addit.Contam.*, **1987**, *5*, 77–83.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL 500 mg Bond-Elut C8 SPE cartridge with 6 mL MeOH, 6 mL water, and 2 mL buffer A. Condition a 6 mL SPE cartridge containing 3 g wet XAD-2 resin with 10 mL MeOH, 10 mL water, and 2 mL buffer B. Homogenize (Ultra-Turrax) 2 g tissue with 20 mL succinate buffer for 1 min, centrifuge at 30 897 g for 15 min, filter (Whatman No. 1 paper) the supernatant, dilute 12 mL filtrate with 6 mL buffer B. For sheep liver add the diluted filtrate to the C8 SPE cartridge, wash with 10 mL buffer A, wash with 2 mL water, elute with 6 mL MeOH. For cow kidney add the diluted filtrate to the XAD-2 cartridge, wash with 14 mL buffer A, wash with 2 mL water, elute with 6 mL MeOH. Inject 25 μL 50 mg/mL copper sulfate and 500 μL water onto column A then load 1.5 mL of the eluate from the SPE cartridge at 0.36 mL/min onto column A. Wash to waste with 500 μL water, 500 μL MeOH, and 500 μL water then elute the contents of column A onto column B with mobile phase A. After 11 min remove column A from the circuit and elute column B with a linear gradient of A:B from 100:0 to 0:100 over 10 min, maintain at 0:100 for 10 min, re-equilibrate to 100:0.

Monitor the effluent from column B. (Buffer A was 100 mM KH_2PO_4 containing 3 g/L pentanesulfonic acid. Succinate buffer was 60 g succinic acid in 1 L water adjusted to pH 4.0 with 1 M NaOH. Buffer B was 37.2 g disodium EDTA and 3 g pentanesulfonic acid in 1 L succinate buffer.)

HPLC VARIABLES
Column: A 10 × 6 10 μm Anagel-TSK-Chelate-SPW (Anachem); B 5 × 3 5 μm Polymer Labs. PLRP-S + 150 × 4.6 5 μm Polymer Labs. PLRP-S
Mobile phase: A was buffer. B was MeCN:MeOH:buffer 25:10:65. (Buffer was 100 mM KH_2PO_4 containing 10 mM citric acid and 10 mM EDTA.)
Injection volume: 1500
Detector: UV 350

CHROMATOGRAM
Retention time: 23
Limit of detection: 10 μg/kg

OTHER SUBSTANCES
Extracted: chlortetracycline, demeclocycline, oxytetracycline

KEY WORDS
SPE; sheep; cow; liver; kidney; column-switching

REFERENCE
Stubbings,G.; Tarbin,J.A.; Shearer,G. On-line metal chelate affinity chromatography clean-up for the high-performance liquid chromatographic determination of tetracycline antibiotics in animal tissues, *J.Chromatogr.B*, **1996**, *679*, 137–145.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL 500 mg C18 SPE cartridge with 20 mL MeOH and 20 mL water. Mix 5 g tissue with 20 mL buffer, homogenize for 30 s, rinse probe twice with 2 mL buffer into the centrifuge tube. Shake for 10 min at high speed and centrifuge at 2500 g for 10 min. Remove the supernatant. Add 20 mL buffer to the tissue plug, shake for 10 min at high speed and centrifuge at 2500 g for 10 min. Remove the supernatant and repeat all steps as described above with 10 mL buffer. Combine the supernatants from all three extractions, centrifuge at 2500 g for 20 min, filter (GF/B paper). Rinse the centrifuge tube twice with 2 mL portions of buffer and filter. Add the filtrate to the SPE cartridge, rinse the flask twice with buffer and add the rinses to the SPE cartridge, wash with 20 mL water, dry the cartridge by drawing air through it, elute with 6 mL 1.26 g/L oxalic acid dihydrate in MeOH, dilute the eluate to 10 mL with water, filter, inject a 60 μL aliquot. (Prepare the buffer (McIlvaine-EDTA buffer) as follows. Mix 1 L 21.0 g/L citric acid monohydrate with 625 mL 28.4 g/L disodium hydrogen phosphate, adjust pH to 4.0 with 100 mM HCl or 100 mM NaOH, add 60.5 g disodium EDTA dihydrate, mix until the solid dissolves.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm C8
Mobile phase: MeCN:MeOH:10 mM oxalic acid 15:20:65
Flow rate: 1.5
Injection volume: 10-60
Detector: UV 350

CHROMATOGRAM
Retention time: 5.9
Limit of detection: 1.5 ng

OTHER SUBSTANCES
Extracted: chlortetracycline, tetracycline

KEY WORDS
SPE; pig; muscle; cow

REFERENCE
MacNeil,J.D.; Martz,V.K.; Korsrud,G.O.; Salisbury,C..C.; Oka,H.; Epstein,R.L.; Barnes,C.J. Chlortetracycline, oxytetracycline, and tetracycline in edible animal tissues, liquid chromatographic method: Collaborative study, *J.AOAC Int.*, **1996**, *79*, 405–417.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Bond Elut C18 SPE cartridge with saturated aqueous disodium EDTA. Blend 5 g tissue with two 20 mL portions and one 10 mL portion of 100 mM pH 4.0 disodium EDTA-McIlvaine buffer at high speed, centrifuge at 850 g for 5 min each time. Combine the supernatants, centrifuge at 850 g for 15 min, filter. Add the filtrate to the SPE cartridge, wash with 20 mL water, air-dry by aspiration for 5 min, elute with 10 mL ethyl acetate followed by 20 mL MeOH:ethyl acetate 5:95, evaporate the eluate to dryness under reduced pressure at 30°, dissolve the residue in 100 μL water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK Gel Super Octyl (Tosoh)
Mobile phase: MeCN:0.05% aqueous trifluoroacetic acid 20:80
Flow rate: 0.5
Injection volume: 50
Detector: MS, Finnigan MAT TSQ 7000 Triple-Stage Quadrupole, electrospray voltage 4.5 kV, gas sheath flow 483 kPa nitrogen, collision gas argon, collision offset -25 V, m/z 445

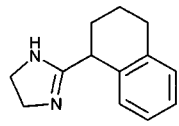
CHROMATOGRAM
Retention time: 4.4

OTHER SUBSTANCES
Extracted: chlortetracycline, doxycycline, oxytetracycline

KEY WORDS
cow; kidney; liver; muscle; SPE

REFERENCE
Oka,H.; Ikai,Y.; Ito,Y.; Hayakawa,J.; Harada,K.-.; Suzuki,M.; Odani,H.; Maeda,K. Improvement of chemical analysis of antibiotics. XXIII. Identification of residual tetracyclines in bovine tissues by electrospray high-performance liquid chromatography-tandem mass spectrometry, *J.Chromatogr.B*, **1997**, *693*, 337–344.

Tetrahydrozoline



Molecular formula: $C_{13}H_{16}N_2$
Molecular weight: 200.28
CAS Registry No.: 84-22-0, 522-48-5 (HCl)
Merck Index: 9358
Lednicer No.: 1 242

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 150 × 6 5 μm Capcell Pak C8 (Shiseido, Japan)
Mobile phase: MeOH:50 mM KH_2PO_4 containing 5 mM tetra-n-butylammonium phosphate 15:85, adjusted to pH 2.6 with 5% orthophosphoric acid (After one week of use, wash the column with water and MeOH:water 70:30 at 1 mL/min for 30 min.)
Column temperature: 30
Flow rate: 1
Injection volume: 10-20
Detector: UV 215

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: chlorpheniramine, dipotassium glycyrrizate, fumaric acid, m-hydroxybenzoic acid, p-hydroxybenzoic acid, maleic acid, neostigmine methylsulfate, pyridoxine, vitamin B12
Noninterfering: chondroitin sulfate, lysozyme

KEY WORDS
ophthalmic solutions; ion-pair agents

REFERENCE
Yamato,S.; Nakajima,M.; Shimada,K. Simultaneous determination of chlorpheniramine and maleate by high-performance liquid chromatography using tetra-n-butylammonium phosphate as an ion-pair reagent, *J.Chromatogr.A*, **1996**, *731*, 346–350.

SAMPLE
Matrix: formulations
Sample preparation: 5 mL Ophthalmic solution + 5 mL 40 μg/mL naphazoline in water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:buffer 30:70 (Buffer was 6 g sodium citrate dihydrate and 4 g anhydrous citric acid in 700 mL water, add 7 mL perchloric acid, adjust pH to 2.2 ± 0.2 with perchloric acid.)
Flow rate: 2
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 5.31
Internal standard: naphazoline (4.37)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
ophthalmic solutions; stability-indicating

REFERENCE
Bauer,J.; Krogh,S. High-performance liquid chromatographic stability-indicating assay for naphazoline and tetrahydrozoline in ophthalmic preparations, *J.Pharm.Sci.*, **1983**, *72*, 1347–1349.

SAMPLE
Matrix: formulations
Sample preparation: Dilute ophthalmic solution with mobile phase to a tetrahydrozoline concentration of 50 μg/mL, filter, mix an aliquot of the filtrate with 100 μg/mL dimethylaminobenzaldehyde in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil C8
Mobile phase: MeCN:MeOH:buffer 5:5:90 (Buffer was 5 mM Na_2HPO_4 containing 5 mM sodium octanesulfonate adjusted to pH 7 with HCl.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 222

CHROMATOGRAM
Retention time: 7

Internal standard: dimethylaminobenzaldehyde (1.3)
Limit of quantitation: 250 ng

OTHER SUBSTANCES
Simultaneous: degradation products, antazoline

KEY WORDS
ophthalmic solutions

REFERENCE
Puglisi,G.; Sciuto,S.; Chillemi,R.; Mangiafico,S. Simultaneous high-performance liquid chromatographic determination of antazoline phosphate and tetrahydrozoline hydrochloride in ophthalmic solution, *J.Chromatogr.*, **1986**, *369*, 165–170.

SAMPLE
Matrix: formulations
Sample preparation: Mix 5 mL nasal solution and 10 mL 500 μg/mL tolazoline hydrochloride in MeOH:water 40:60, make up to 50 mL with MeOH:water 40:60, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.1 RSIL C18 (RSL, Eke, Belgium)
Mobile phase: MeOH:water 40:60 containing 20 mM sodium 1-octanesulfonate and 10 mM N,N-dimethyloctylamine, pH adjusted to 3.0 with orthophosphoric acid
Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: 3.5
Internal standard: tolazoline (2.5)

OTHER SUBSTANCES
Simultaneous: degradation products, antazoline, coumazoline, lidocaine, naphazoline, oxymetazoline, prednisolone, sulfadimidine, sulfanilamide, sulfathiazole, tenaphtoxaline, tramazoline, xylometazoline

KEY WORDS
nasal solutions; stability-indicating

REFERENCE
De Schutter,J.A.; Van den Bossche,W.; De Moerloose,P. Stability-indicating analysis of tetryzoline hydrochloride in pharmaceutical formulations by reversed-phase ion-pair liquid chromatography, *J.Chromatogr.*, **1987**, *391*, 303–308.

SAMPLE
Matrix: formulations
Sample preparation: 2 mL Sample + 1 mL 200 μg/mL emetine hydrochloride in water, make up to 10 mL with mobile phase, filter (0.45 μm), inject a 50-100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Technosphere RP C-8 (HPLC Technology)
Mobile phase: MeCN:40 mM tetramethylammonium bromide:1 M acetic acid 80:15:5 (apparent pH 4.5)
Flow rate: 1.5
Injection volume: 50-100
Detector: UV 260

CHROMATOGRAM
Retention time: 1.43

Internal standard: emetine (1.83)
Limit of quantitation: 50 μg/mL

OTHER SUBSTANCES
Simultaneous: benzalkonium (C12, C14, C16)
Interfering: naphazoline

KEY WORDS
nasal; ophthalmic solutions

REFERENCE
Santoni,G.; Medica,A.; Gratteri,P.; Furlanetto,S.; Pinzauti,S. High-performance liquid chromatographic determination of benzalkonium and naphazoline or tetrahydrozoline in nasal and ophthalmic solutions, *Farmaco*, **1994**, *49*, 751–754.

SAMPLE
Matrix: solutions

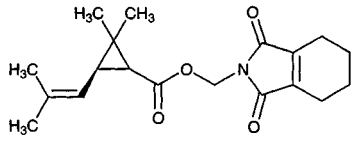
HPLC VARIABLES
Column: 250 × 4.6 Chirex 3017 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 50:35:15 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 1
Injection volume: 20
Detector: UV 242

CHROMATOGRAM
Retention time: 10.5, 12.5 (enantiomers)

KEY WORDS
chiral

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

Tetramethrin



Molecular formula: $C_{19}H_{25}NO_4$
Molecular weight: 331.41
CAS Registry No.: 7696-12-0
Merck Index: 9362

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 209.9

CHROMATOGRAM
Retention time: 3.233

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: fruit, vegetables
Sample preparation: Prepare a cleanup column by placing 4 g Florisil, 1 g activated charcoal, and a 20 mm layer of anhydrous sodium sulfate in a 400 × 10 glass column, wash with 40 mL toluene, wash with 40 mL toluene:MeCN 99:1. Homogenize 25 g chopped fruit or vegetable with 70 mL MeOH at high speed for 3 min, filter, homogenize solid with 30 mL MeOH, filter. Combine the filtrates and add them to 60 mL toluene and 300 mL 10% NaCl in water, shake well for 3 min, let layers separate. Dry the organic layer by passing it through 20 g anhydrous sodium sulfate in a 20 mm diameter column, concentrate to about 5 mL under reduced pressure at 80°, add to the cleanup column, elute with 40 mL toluene:MeCN 99:1. Evaporate the eluate just to dryness under reduced pressure at 80°, reconstitute with 1 mL MeOH, inject an aliquot. (Reflux activated charcoal (20-40 mesh) with 1 M HCl for 4 h, wash with water until the washings are neutral, dry at 95-100° (J.Assoc.Off.Anal.Chem. 1983, 66, 1013). Heat 60-100 mesh Florisil at 200° for 24 h, cool, add 4% water, mix thoroughly, store in a sealed jar (J.Assoc.Off.Anal.Chem. 1983, 66, 1003).)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. MeCN:water from 62:38 to 78:22 over 32 min (Waters curve 6).
Column temperature: 50
Flow rate: 1.5
Detector: UV 206

CHROMATOGRAM
Retention time: 13.08
Limit of detection: 50 ng/g

OTHER SUBSTANCES
Simultaneous: allethrin, biphenthrin, cypermethrin, fenpropathrin, fenvalerate, flucythrinate, methothrin, permethrin, Py-115

KEY WORDS
cucumber; tomato; cabbage; apple; pear; peach; SPE

REFERENCE
Pang,G.-F.; Chao,Y.-Z.; Liu,X.-S.; Fan,C.-L. Multiresidue liquid chromatographic method for simultaneous determination of pyrethroid insecticides in fruits and vegetables, *J.AOAC Int.*, **1995**, *78*, 1474–1480.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 50 × 4 40 μm pellicular material
Column: 250 × 4.6 5 μm silica (IBM)
Mobile phase: Hexane:isopropanol 98:2
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 3.90 (cis), k' 4.15 (trans)

OTHER SUBSTANCES
Also analyzed: allethrin, chrysanthemol, dimethrin, ethyl chrysanthemate, cyfluthrin (baythroid), permethrin, phenothrin, resmethrin, RU-11679

KEY WORDS
normal phase

REFERENCE
Abidi,S.L. Column selectivity in high-performance liquid chromatography of substituted *gem*-dimethylcyclopropanes, *J.Chromatogr.*, **1986**, *368*, 59–76.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 50 × 4 40 μm pellicular material
Column: 250 × 4.6 5 μm β-cyclodextrin-bonded silica (7.4 mequivalents cyclodextrin per gram of silica) (Advanced Separation Technologies)
Mobile phase: MeOH:water 60:40
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 10.0 (+, cis), k' 10.4 (-, cis), k' 17.8 (+, trans), k' 20.4 (-, trans)

OTHER SUBSTANCES
Also analyzed: allethrin, cyfluthrin (baythroid), chrysanthemol, dimethrin, ethyl chrysanthemate, permethrin, phenothrin, resmethrin, RU-11679

KEY WORDS
chiral

REFERENCE
Abidi,S.L. Column selectivity in high-performance liquid chromatography of substituted *gem*-dimethylcyclopropanes, *J.Chromatogr.*, **1986**, *368*, 59–76.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 0.1-1 mg/mL solution in hexane.

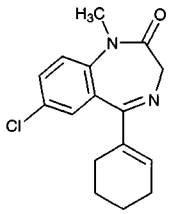
HPLC VARIABLES
Guard column: 5 μm Spherisorb NH2
Column: 250 × 4.6 Pirkle ionic type 1-A column (Technicol)
Mobile phase: Hexane:isopropanol 99.85:0.15
Flow rate: 2.5
Detector: UV 230

OTHER SUBSTANCES
Also analyzed: allethrin, cypermethrin, fenpropathrin, fenvalerate

KEY WORDS
chiral

REFERENCE
Lisseter,S.G.; Hambling,S.G. Chiral high-performance liquid chromatography of synthetic pyrethroid insecticides, *J.Chromatogr.*, **1991**, *539*, 207–210.

Tetrazepam



Molecular formula: $C_{16}H_{17}ClN_2O$
Molecular weight: 288.78
CAS Registry No.: 10379-14-3
Merck Index: 9379

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 227

CHROMATOGRAM
Retention time: 8.08
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazo-

lam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 226.3

CHROMATOGRAM

Retention time: 22.378

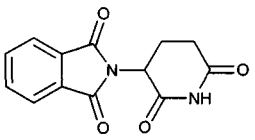
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Thalidomide



Molecular formula: $C_{13}H_{10}N_2O_4$
Molecular weight: 258.23
CAS Registry No.: 50-35-1
Merck Index: 9390
Lednicer No.: 1 257

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: chiral protein (avidin) column
Mobile phase: 2-propanol:1 M pH 4.0 phosphate buffer 2:98
Injection volume: 50
Detector: UV 300

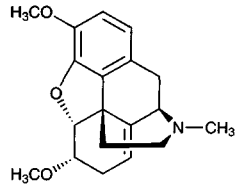
CHROMATOGRAM
Internal standard: labetalol
Limit of detection: 50 ng/ml
Limit of quantitation: 100 ng/ml

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
chiral

REFERENCE
Tata,P.N.V.; Bramer,S.L. Enantiomeric assay of grepafloxacin in plasma (Abstract 4162), *Pharm.Res.*, **1997**, *14*, S684.

Thebaine



Molecular formula: $C_{19}H_{21}NO_3$
Molecular weight: 311.38
CAS Registry No.: 115-37-7
Merck Index: 9411

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

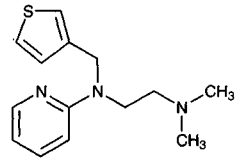
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphet-

amine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Thenyldiamine



Molecular formula: $C_{14}H_{19}N_3S$
Molecular weight: 261.39
CAS Registry No.: 91-79-2
Merck Index: 9416

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

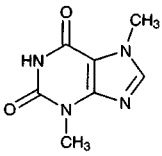
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.3

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Theobromine



Molecular formula: $C_7H_8N_4O_2$
Molecular weight: 180.17
CAS Registry No.: 83-67-0
Merck Index: 9418
Lednicer No.: 1 423

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 204

CHROMATOGRAM
Retention time: 3.79

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

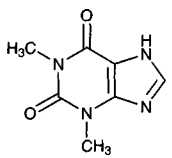
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide,

hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Theophylline



Molecular formula: $C_7H_8N_4O_2$

Molecular weight: 180.17

CAS Registry No.: 58-55-9, 32156-80-2 (diethanolamine), 573-41-1 (ethanolamine), 5600-19-1 (isopropanolamine), 8002-89-9 (sodium acetate), 8000-10-1 (sodium glycinate), 5967-84-0 monohydrate

Merck Index: 9421

Lednicer No.: 1 423; 2 464; 3 230; 4 165, 168, 213

SAMPLE

Matrix: blood

Sample preparation: Mix 50 μL plasma with 50 μL 20 μg/mL IS in water, vortex for 10 s, add 20 μL 20% perchloric acid, vortex for 10 s, centrifuge at 2000 g for 5 min, inject a 50 μL of the supernatant.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb C18
Mobile phase: MeCN:THF:concentrated acetic acid:water 2:2:0.5:95.5
Column temperature: 35
Flow rate: 1
Injection volume: 50
Detector: UV 273

CHROMATOGRAM

Retention time: 5
Internal standard: 7-(β-hydroxypropyl)theophylline (9.2)
Limit of detection: 100 ng/mL

Limit of quantitation: 2 μg/mL

OTHER SUBSTANCES
Extracted: caffeine
Simultaneous: β-hydroxyethyltheophylline, 8-chlorotheophylline, theobromine

KEY WORDS
plasma

REFERENCE
Schreiber-Deturmeny,E.; Bruguerolle,B. Simultaneous high-performance liquid chromatographic determination of caffeine and theophylline for routine drug monitoring in human plasma, *J.Chromatogr.B*, **1996**, *677*, 305–312.

SAMPLE
Matrix: blood
Sample preparation: 190 μL Plasma + 200 μL 10% trichloroacetic acid solution, vortex for 30 s, centrifuge at 3000 g for 10 min. Inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:water adjusted to pH 4 9:91
Flow rate: 1.2
Injection volume: 25-50
Detector: UV 273

CHROMATOGRAM
Limit of detection: 300 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Contreras,J.; Pérez,N.; González,R.; Ontivero,E.; López,M. Single dose study of the bioequivalence of two sustained-release theophylline formulations, *Arzneimittelforschung*, **1998**, *48*, 259–262.

SAMPLE
Matrix: blood
Sample preparation: Inject a 5 μL aliquot of serum directly.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 5:95
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 2.33

OTHER SUBSTANCES
Extracted: acetaminophen

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fntz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood, saliva
Sample preparation: Add 500 μL plasma, serum, or saliva to 200 mg ammonium sulfate, add 50 μL 15 μg/mL IS in water, add 500 μL 200 mM pH 4.5 sodium acetate buffer, vortex briefly, add 3 mL dichloromethane, shake at 85 cycles/min for 10 min, centrifuge at 2000 g for 10min. Evaporate the organic layer to dryness at 40° under a stream of nitrogen, reconstitute in 250 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 pellicular C18
Column: 100 × 4.6 3 μm Microsorb MV C18
Mobile phase: MeOH:THF:100 mM pH 4.5 sodium acetate:water 6.5:1.4:10:82.1
Flow rate: 0.8
Injection volume: 50
Detector: UV 274

CHROMATOGRAM
Retention time: 5.9
Internal standard: β-hydroxyethyltheophylline (6.5)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: caffeine, paraxanthine, theobromine
Noninterfering: chlorzoxazone, dapsone

KEY WORDS
plasma; serum

REFERENCE
Frye,R.F.; Stiff,D.D.; Branch,R.A. A sensitive method for the simultaneous determination of caffeine and its dimethylxanthine metabolites in human plasma: Application to CYP1A2 phenotyping, *J.Liq. Chromatogr.Rel.Technol.*, **1998**, *21*, 1161–1171.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM
Retention time: 4.877

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Weight out powdered tablets, dissolve in 50 mM sodium dodecyl sulfate in an ultrasonic bath. Filter (no.4 sintered glass plate), dilute, inject an aliquot. Capsules. Dissolve capsules in 50 mM sodium dodecyl sulfate in an ultrasonic bath. Drops. Dilute drops with 50 mM sodium dodecyl sulfate. Inject an aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 5 μmC18 (Scharlau, Spain)
Column: 120 × 4.6 5 μm Spherisorb ODS-2 C18
Mobile phase: Propanol:50 mM sodium dodecyl sulfate 3:97, adjusted to pH 7 with 10 mM phosphate buffer
Flow rate: 1
Injection volume: 20
Detector: UV 273

CHROMATOGRAM
Retention time: 4

KEY WORDS
tablets; capsules; drops

REFERENCE
Perez-Martinez,I.; Sagrado,S.; Medina-Hernández,M.J. Determination of theophylline in pharmaceuticals by micellar liquid chromatography and spectrophotometric detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1957–1966.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 18:82
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 275

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 5 μm 3-aminopropylsilyl silica gel with the amino group derivatized with 1,8-naphthalic anhydride (Bunseki Kagaku 1993, 42, 817)
Mobile phase: MeOH:buffer 50:50 (Prepare buffer by dissolving 6.183 g boric acid and 1.461 g NaCl in 500 mL water, adjust pH to 6.4 with sodium borate solution.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 13.5
Internal standard: 1,3-dimethyl-7-(2-hydroxyethyl)xanthine (12)

OTHER SUBSTANCES
Simultaneous: caffeine, hypoxanthine, pentoxifylline, propentofylline, theobromine, uric acid, xanthine

REFERENCE
Nakashima,K.; Inoue,K.; Mayahara,K.; Kuroda,N.; Hamachi,Y.; Akiyama,S. Use of 3-(1,8-naphthalimido)propyl-modified silyl silica gel as a stationary phase for the high-performance liquid chromatographic separation of purine derivatives, *J.Chromatogr.A*, **1996**, *722*, 107–113.

SAMPLE
Matrix: urine
Sample preparation: Add 100-120 mg NaCl, 50 μL 100 μg/mL β-hydroxyethyltheophylline, and 100 μL ammonia buffer to 1 mL urine. Extract with 5 mL MeOH:dichloromethane 10:90 for 10 min, centrifuge at 150 g for 5 min, remove the upper aqueous layer and evaporate the organic layer under nitrogen at 40°. Dissolve the residue in 200 μL mobile phase, inject a 20 μL aliquot. (Prepare the pH 9.5 ammonia buffer by adding ammonia to a saturated ammonium chloride solution.)

HPLC VARIABLES
Guard column: 10 × 2 40 μm C18
Column: 100 × 3 5 μm Hypersil 5 ODS (Chrompack)
Mobile phase: THF:water 1:100
Flow rate: 1
Injection volume: 20
Detector: UV 275

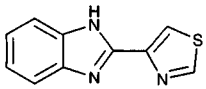
CHROMATOGRAM
Retention time: 3.6
Internal standard: β-hydroxyethyltheophylline (4.5)
Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES
Extracted: caffeine, theobromine, paraxanthine

KEY WORDS
horse; human; urine

REFERENCE
Delbeke,F.T.; De Backer,P. Threshold level for theophylline in doping analysis, *J.Chromatogr.B*, **1996**, *687*, 247–252.

Thiabendazole



Molecular formula: $C_{10}H_7N_3S$
Molecular weight: 201.25
CAS Registry No.: 148-79-8
Merck Index: 9426
Lednicer No.: 1 325

SAMPLE
Matrix: abomasal fluid, blood, duodenal fluid, rumen fluid
Sample preparation: 4 mL Plasma, rumen fluid, abomasal fluid, or duodenal fluid + 4 mL pH 7.4 phosphate buffer + 20 mL ether, shake on a rotary mixer for 10 min, remove 16 mL of the

ether layer, add 20 mL ether, shake on a rotary mixer for 10 min, remove 20 mL of the ether layer. Combine the ether layers and evaporate them under a stream of nitrogen at 60° to dryness, reconstitute in 50 μL MeOH, sonicate, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 ODS Hypersil 10
Mobile phase: MeOH:50 mM ammonium carbonate 65:35
Flow rate: 1.5
Injection volume: 5
Detector: UV 292

CHROMATOGRAM
Retention time: 3.8
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: albendazole, oxfendazole, cambendazole, mebendazole, oxibendazole, fenbendazole, parbendazole

KEY WORDS
plasma; sheep

REFERENCE
Bogan,J.A.; Marriner,S. Analysis of benzimidazoles in body fluids by high-performance liquid chromatography, *J.Pharm.Sci.*, **1980**, *69*, 422–423.

SAMPLE
Matrix: food
Sample preparation: Blend 10 g food and 20 mL MeOH in a Polytron at high speed for 3 min. Centrifuge at 5000 g for 10 min, inject a 50 μL aliquot. Alternatively, shake 5 or 10 g food, 20 mL MeOH, and 5 ball bearings for 10 min. Remove a 1 mL aliquot, centrifuge at 5000 g for 10 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: Ultracarb 30 ODS
Mobile phase: MeCN:MeOH:water:monoethanolamine 36.4:13.6:50:0.02 (A) or 31.32:8.44:60.24:0.01 (B)
Flow rate: 1.0
Injection volume: 50
Detector: F ex 305 em 345

CHROMATOGRAM
Retention time: 3.5 (A), 5.7 (B)
Limit of quantitation: 6 ppb

KEY WORDS
fruits; vegetables

REFERENCE
Rushway,B.J.; Perkins,L.B.; Larkin,K.L.; Fan,T.S. A modified high performance liquid chromatographic analysis of thiabendazole in fruits and vegetables with ELISA confirmation, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1217–1226.

SAMPLE
Matrix: food
Sample preparation: Place 25 g homogenized potato (washed or peeled) in a separating funnel, add 20 mL saturated aqueous NaCl solution and extract with three 50 mL portions of dichloromethane. Dry organic layers over anhydrous sodium sulfate and evaporate to dryness under reduced pressure. Reconstitute the residue in 1 mL dichloromethane and spot four 400 μL portions of this solution on a 200 × 200 mm × 250 μm thick silica 60 TLC plate (Merck). Develop with chloroform:triethylamine 100:5 then with MeOH:chloroform 5:100 (Caution!

Chloroform is a carcinogen!), remove the area containing thiabendazole and shake with 400 μL MeOH, centrifuge. Dilute solution to 2.5 mL with MeOH, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC18
Mobile phase: MeOH:100 mM pH 6.6 phosphate buffer 60:40
Flow rate: 2
Injection volume: 6
Detector: UV 248

CHROMATOGRAM
Retention time: 1.8
Limit of detection: 500 pg/g
Limit of quantitation: 1.7 ng/g

OTHER SUBSTANCES
Extracted: propham, chlorpropham

KEY WORDS
comparison with HPTLC; potato

REFERENCE
Corti,P.; Dreassi,E.; Politi,N.; Aprea,C. Comparison of an HPTLC and an HPLC procedure for the determination of chlorpropham, propham and thiabendazole residues in potatoes, *Food Addit.Contam.*, **1991**, *8*, 607–615.

SAMPLE
Matrix: food
Sample preparation: Raw potatoes. Weigh out 23.0-25.5 g potato peelings or flesh macerated in a domestic food processor (Magimix 4000) for 1 min, homogenize with 60 mL dichloromethane and 70 g anhydrous sodium sulfate (Silverson mixer-emulsifier) for 1 min. Decant the supernatant through glass fiber filter (Whatman GF/A), repeat extraction with three 40 mL portions of fresh dichloromethane, filter homogenate through a suction Buchner funnel, wash with two 15 mL portions of dichloromethane. Dry the combined filtrate and washings with anhydrous sodium sulfate, re-filter and evaporate to near dryness on a rotary evaporator at 35°. Dissolve the concentrate in 100-200 mL MeOH (peelings), or 5-25 mL MeOH (flesh), filter the extract through a 0.45 μm nylon membrane (Micron Separation Inc.) and inject an aliquot. Baked potatoes. Mix 29.89-51.85 g baked peelings or 50 g baked flesh with 80-100 g anhydrous sodium sulfate before maceration for 2 min. Homogenize with 80 mL dichloromethane for 2 min, decant the supernatant through a glass fiber filter (Whatman GF/A), repeat extraction with two 70 mL portions of fresh dichloromethane, filter homogenate through a suction Buchner funnel, wash with two 15 mL portions of dichloromethane. Dry the combined filtrate and washings with anhydrous sodium sulfate, re-filter and evaporate to near dryness on a rotary evaporator at 35°. Dissolve the concentrate in 100-200 mL MeOH (peelings), or 5-25 mL MeOH (flesh), filter extract through a 0.45 μm nylon membrane (Micron Separation Inc.) and inject an aliquot.

HPLC VARIABLES
Guard column: 4 × 4 7 μm LiChrosorb RP Select B (Merck)
Column: 125 × 4 7 μm LiChrosorb RP Select B (Merck)
Mobile phase: MeCN:ammonia solution (d 0.88):water 55:0.27:45
Flow rate: 1
Injection volume: 20
Detector: UV 303, F ex 296 em 351

CHROMATOGRAM
Retention time: 1.68 (UV), 1.94 (F)

KEY WORDS
potatoes

REFERENCE
Friar,P.M.; Reynolds,S.L. The effects of microwave-baking and oven-baking on thiabendazole residues in potatoes, *Food Addit.Contam.*, **1991**, *8*, 617–626.

SAMPLE
Matrix: food
Sample preparation: Condition a 3 mL 500 mg Sep-Pak Vac Silica SPE cartridge with 10 mL MeOH and 10 mL ethyl acetate. Condition a 3 mL 300 mg Bond Elut PRS cartridge with 10 mL water and 10 mL MeOH. Place the Sep Pak Vac Silica cartridge on top of the Bond Elut PRS cartridge. Citrus fruit. Slice and homogenize citrus fruit with a mixer. 5 g Aliquot of sample + 20 g anhydrous sodium sulfate + 1.5 g anhydrous sodium hydrogen phosphate + 30 mL ethyl acetate, blend at high-speed, centrifuge at 3100 rpm for 8 min, remove the supernatant. Re-extract with 20 mL ethyl acetate, combine the supernatants. Add the crude extract to the double SPE cartridge, allow to pass at ca. 1 mL/min, wash with 5 mL ethyl acetate, remove the Sep-Pak Vac Silica cartridge. Wash the second SPE cartridge with 10 mL MeOH and 10 mL 100 mM NaCl, elute with 10 mL mobile phase, inject a 20 μL aliquot. Banana. 10 g Homogenized sample + 40 g anhydrous sodium sulfate + 50 mL ethyl acetate, extract as described above. Re-extract with 30 mL ethyl acetate, combine the supernatants. Add the crude extract to the double SPE cartridge, allow to pass at ca. 1 mL/min, wash with 5 mL ethyl acetate, remove the Sep-Pak Vac Silica cartridge. Wash the second SPE cartridge with 10 mL MeOH and 10 mL 100 mM NaCl, elute with 10 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSKgel ODS-80Ts (TOSOH, Japan)
Mobile phase: MeCN:MeOH:water 40:30:30 containing 10 mM sodium 1-tridecanesulfonate, adjusted to pH 2.5 with phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 100 ng/g (citrus fruit), 50 ng/g (banana)

OTHER SUBSTANCES
Extracted: enilconazole

KEY WORDS
citrus fruit; banana; SPE

REFERENCE
Ito,Y.; Ikai,Y.; Oka,H.; Hayakawa,J.; Kagami,T. Application of ion-exchange cartridge clean-up in food analysis. I. Simultaneous determination of thiabendazole and imazalil in citrus fruit and banana using high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.A*, **1998**, *810*, 81–87.

SAMPLE
Matrix: fruit
Sample preparation: Condition a 10 mL 500 mg Bond-Elut PRS LRC cartridge with 10 mL MeOH:water 80:20 containing 1% phosphoric acid, 3 mL MeOH, and 5 mL ethyl acetate. Homogenize unwashed, divided citrus fruit with dry ice. Mix 5 g citrus homogenate with 5 mL pH 8 buffer (Fisher Scientific). Adjust the pH to about 8.0 with 200 mM NaOH. Add 25 mL ethyl acetate, shake for 15 min, centrifuge at 2500 rpm for 15 min. Mix a 15-20 mL aliquot of the ethyl acetate extract with 4 g anhydrous sodium sulfate, shake manually for 5 s, add more sodium sulfate if the ethyl acetate is not clear. Add 10 mL of the dry extract to the SPE cartridge, wash with 5 mL ethyl acetate, air dry the SPE cartridge under vacuum, elute with 9.8 mL elution solution, dilute the eluate to 10 mL with elution solution, inject a 50 μL aliquot. (Elution solution was 100 mM KH_2PO_4 in MeCN:water 70:30.)

HPLC VARIABLES
Column: 125 × 4.6 5 μm PartiSphere SCX (Whatman)

Mobile phase: MeOH:buffer 25:75 (Prepare buffer as follows. Dissolve 13.6 g KH_2PO_4 in 1 L water, add 500 mL MeCN, shake, dilute to 2 L with water. Adjust pH to about 3.4 with phosphoric acid.)
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: F ex 305 em 380

CHROMATOGRAM
Retention time: 4.3
Limit of quantitation: 0.05 ppm

KEY WORDS
SPE; citrus

REFERENCE
Arenas,R.V.; Rahman,H.; Johnson,N.A. Determination of thiabendazole residues in whole citrus fruits by liquid chromatography with fluorescence detection, *J.AOAC Int.*, **1996**, *79*, 579–582.

SAMPLE
Matrix: fruit, juice, vegetables
Sample preparation: 5 g Fruit, juice, or vegetables or 2 g bulk juice or 0.5 g dried sample + 20 mL solvent + 20 mL dichloromethane, homogenize (Brinkmann polytron) at medium speed for 2 min, shake rapidly by hand for 3 min, centrifuge at 5000 g for 3 min. Remove the dichloromethane layer and dry it over 0.5 g anhydrous sodium sulfate. Remove a 10 mL aliquot and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, centrifuge at 10000 g for 5 min, inject a 10 μL aliquot. (Solvent was 5 mL EtOH + 15 mL 2 M ammonium chloride adjusted to pH 9.5 with 14.5 M ammonium hydroxide.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultracarb 30 ODS (Phenomenex)
Mobile phase: MeCN:MeOH:water:monoethanolamine 26:7:50:0.01
Flow rate: 1
Injection volume: 10
Detector: F ex 305 em 345

CHROMATOGRAM
Retention time: 5
Limit of quantitation: 0.04 ng

KEY WORDS
banana; orange; grapefruit; pear; apple; potato; raspberry; cranberry; grape; lime

REFERENCE
Bushway,R.J.; Li,L.; Paradis,L.R.; Perkins,L.B. Determination of thiabendazole in potatoes, fruits, and their processed products by liquid chromatography, *J.AOAC Int.*, **1995**, *78*, 815–820.

SAMPLE
Matrix: fruit, vegetables
Sample preparation: 50 g Homogenized sample + 100 mL MeOH, shake mechanically for 10 min, filter (shark skin paper), rinse solid with 50 mL MeOH. Add 10 mL 1 M HCl and 100 mL 1% NaCl to the filtrate, add 100 mL dichloromethane, shake for 1 min, repeat extraction. Combine the extracts and evaporate just to dryness under reduced pressure below 30°, reconstitute the residue in 4 mL MeOH, add 6 mL buffer, mix, filter (0.45 μm), inject a 25 μL aliquot (minor part). Rinse aqueous/MeOH layer into beaker with two 10 mL portions of water, adjust pH to 7.5-8 with NaOH or HCl, extract twice with 100 mL portions of dichloromethane. Combine the extracts and evaporate just to dryness under reduced pressure below 30°, reconstitute the residue in 4 mL MeOH, add 6 mL buffer, mix, filter (0.45 μm), inject a 25 μL aliquot (major part). (Buffer was 1 g sodium 1-decanesulfonate in 200 mL water, 7 mL phosphoric acid, and 10 mL triethylamine, make up to 1 L with water.)

HPLC VARIABLES
Guard column: Supelguard LC-18-DB (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-18-DB or Ultrasphere C-18 IP
Mobile phase: MeOH:buffer 35:65 (Buffer was 1 g sodium 1-decanesulfonate in 200 mL water, 7 mL phosphoric acid, and 10 mL triethylamine, make up to 1 L with water.)
Column temperature: 40
Flow rate: 1.5
Injection volume: 25
Detector: UV 305 or F ex 305 em 345

CHROMATOGRAM
Retention time: 20
Limit of quantitation: 10 ppb (F)

OTHER SUBSTANCES
Extracted: allophanate, benomyl, thiophanate methyl

KEY WORDS
apples; bananas; lemons; peaches; pineapples; peas; rice

REFERENCE
Gilvydis,D.M.; Walters,S.M. Ion-pairing liquid chromatographic determination of benzimidazole fungicides in foods, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 753–761.

SAMPLE
Matrix: milk
Sample preparation: Condition a 3 mL Bond Elut silica SPE cartridge with 2 mL dichloromethane. 10 g Milk + 5 mL 1 M sodium carbonate, mix, add 150 mL ethyl acetate, add 1 mL 10 mg/mL BHT in ethyl acetate, blend (tissuemizer) at high speed for 5 min, add 10 g anhydrous sodium sulfate, blend for 1 min, let settle for 2-3 min, filter (No. 41 paper), add another 150 mL ethyl acetate to the sodium sulfate, blend for 2 min, filter. Combine the filtrates and evaporate them to dryness under vacuum. Rinse out flask with two 10 mL portions of hexane and two 10 mL portions of 1 M phosphoric acid. Combine rinses, shake vigorously for 2 min, extract the hexane layer with two 10 mL portions of 1 mL phosphoric acid. Combine all the aqueous layers and wash them with 5 mL hexane, adjust the pH to 8-9 by slowly adding about 9 mL 10 M KOH (use an ice bath), add 50 mL ethyl acetate, shake vigorously for 2 min, repeat extraction. Filter the organic layers through 40 g anhydrous sodium sulfate, wash the sodium sulfate with 25 mL ethyl acetate. Combine the organic layers, add 200 μL 10 mg/mL BHT in ethyl acetate, evaporate to dryness under vacuum. Take up the residue in two 3 mL portions of dichloromethane and add them to the SPE cartridge, wash with 5 mL dichloromethane, elute with 5 mL dichloromethane:MeOH 75:25. Evaporate the eluate to dryness under nitrogen, reconstitute in 1 mL mobile phase, vortex, filter (0.2 μm), inject an aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 pellicular C18 (Alltech)
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:buffer 53:47 (Buffer was 1.15 g $(NH_4)H_2PO_4$ in 950 mL water, adjust pH to 7.0 with dilute ammonia, make up to 1 L with water.)
Flow rate: 1
Injection volume: 50
Detector: UV 298

CHROMATOGRAM
Retention time: 17
Limit of detection: 0.5 ppb

OTHER SUBSTANCES
Extracted: oxfendazole

KEY WORDS
cow; SPE

REFERENCE
Tai,S.S.; Cargile,N.; Barnes,C.J. Determination of thiabendazole, 5-hydroxythiabendazole, fenbendazole, and oxfendazole in milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 368–373.

SAMPLE
Matrix: milk
Sample preparation: Condition a 500 mg 2.8 mL Regular Bond Elut PRS (propylsulfonic acid) SPE cartridge with 10 mL 1% phosphoric acid in MeOH:water 80:20, with 3 mL MeOH, and with 5 mL ethyl acetate. Heat 5 g milk + 2.5 mL concentrated HCl at 85-90° for 4 h, cool to room temperature, add 5 mL 6 M NaOH, shake, cool to room temperature, adjust the pH to 8.0 with 6 M and 0.2 M NaOH, add 5 mL buffer, add 20 mL ethyl acetate, shake on a reciprocating shaker for 15 min, centrifuge at 3200 g for 5 min, add the ethyl acetate layer to the SPE cartridge, repeat the extraction, add this ethyl acetate layer to the SPE cartridge, wash with 5 mL ethyl acetate. Air dry the SPE cartridge under vacuum, elute with 9.5 mL 100 mM KH_2PO_4 in MeCN:water 30:70, collect the eluate and make up to 10 mL with 100 mM KH_2PO_4 in MeCN:water 30:70, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm PartiSphere SCX benzenesulfonic acid (Whatman)
Mobile phase: MeCN:8.5 g/L KH_2PO_4 20:80, adjust the pH to 3.8 with phosphoric acid
Column temperature: 25
Flow rate: 1
Injection volume: 50
Detector: F ex 305 em 380

CHROMATOGRAM
Retention time: 9.0
Limit of quantitation: 50 ppb

OTHER SUBSTANCES
Extracted: metabolites, 5-hydroxythiabendazole (F ex 318 em 525)

KEY WORDS
cow; SPE

REFERENCE
Arenas,R.V.; Johnson,N.A. Liquid chromatographic fluorescence method for multiresidue determination of thiabendazole and 5-hydroxythiabendazole in milk, *J.AOAC Int.*, **1995**, *78*, 642–646.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin,

cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Inject 50 μL of a solution in RPMI-1640.

HPLC VARIABLES

Column: 250 × 4.6 Hypersil ODS
Mobile phase: MeOH:37 mM NaH_2PO_4 60:40 adjusted to pH 7.5 with triethylamine
Injection volume: 50
Detector: UV 298

CHROMATOGRAM

Retention time: 6.0

REFERENCE

Ho,N.F.H.; Sims,S.M.; Vidmar,T.J.; Day,J.S.; Barsuhn,C.L.; Thomas,E.M.; Geary,T.G.; Thompson,D.P. Theoretical perspectives on anthelmintic drug discovery: Interplay of transport kinetics, physicochemical properties, and in vitro activity of anthelmintic drugs, *J.Pharm.Sci.*, **1994**, *83*, 1052–1059.

SAMPLE

Matrix: solutions
Sample preparation: Centrifuge at 10000 rpm, dilute the supernatant with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil MOS
Mobile phase: MeCN:10 mM KH_2PO_4 60:40
Detector: UV 254

REFERENCE
Okimoto,K.; Rajewski,R.A.; Uekama,K.; Jona,J.A.; Stella,V.J. The interaction of charged and uncharged drugs with neutral (HP-β-CD) and anionically charged (SBE7-β-CD) β-cyclodextrins, *Pharm.Res.*, **1996**, *13*, 256–264.

SAMPLE
Matrix: tissue
Sample preparation: Wash 22 g bulk 40 μm 18% load end-capped C18 material (Analytichem) in a syringe barrel with 100 mL hexane, with 100 mL dichloromethane, and with 100 mL MeOH and dry under vacuum aspiration. Gently blend 2 g C18 material, 0.5 g liver, and 10 μL 40 μg/mL mebendazole in DMF in a glass pestle for 1 min until homogeneous in appearance. Place in a 10 mL syringe barrel plugged with filter paper (Whatman No. 1), cover with filter paper, compress to 4.5 mL, place a 100 μL pipette tip on the barrel to restrict flow, wash with 8 mL hexane, elute with 8 mL MeCN. Pass the eluate through 0.5 g activated alumina (EM Science Type F-20 80-200 mesh) between filter paper in a 10 mL syringe barrel (wash column with 4 mL MeCN just before use). Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH and 400 μL 17 mM phosphoric acid, sonicate for 5-10 min, centrifuge at 17000 g for 5 min, filter the supernatant (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm Micro Pak ODS (Varian)
Mobile phase: MeCN:17 mM phosphoric acid 40:60
Column temperature: 45
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 5
Internal standard: mebendazole (9)
Limit of detection: 100 ng/g

OTHER SUBSTANCES
Extracted: albendazole, oxfendazole, fenbendazole

KEY WORDS
matrix solid-phase dispersion; liver

REFERENCE
Long,A.R.; Malbrough,M.S.; Hsieh,L.C.; Short,C.R.; Barker,S.A. Matrix solid phase dispersion isolation and liquid chromatographic determination of five benzimidazole anthelmintics in fortified beef liver, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 860–863.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 2.8 mL 500 mg 40 μm 60 Å Bond Elut silica SPE cartridge with 2 mL dichloromethane. 10 g Minced tissue + 5 mL 1 M sodium carbonate + 150 mL ethyl acetate + 1 mL 10 mg/mL BHT in ethyl acetate, blend (Waring) at high speed for 5 min, add 80 g anhydrous sodium sulfate, blend at low speed for 1 min. Decant the organic layer and filter it (No. 41 paper), add 150 mL acetone to material remaining in blender, blend at low speed for 2-3 min, filter, wash solid with 10 mL EtOH. Combine all the filtrates and evaporate them to dryness under vacuum at 30-35° (beware of bumping). Rinse out flask with two 10 mL portions of hexane and two 10 mL portions of 1 M phosphoric acid, combine rinses, shake vigorously for 2 min, allow to separate for 10 min, extract the hexane layer twice more with 10 mL portions of 1 M phosphoric acid. Combine all the aqueous layers and wash them with 10 mL hexane, adjust the pH of the aqueous layer to 8.5 ± 1.0 by slowly adding about 9 mL 10 M KOH while using an ice bath. Extract twice with 50 mL ethyl acetate (2 min shaking),

pass ethyl acetate layers through 40 g anhydrous sodium sulfate, wash the sodium sulfate with 25 mL ethyl acetate. Combine the ethyl acetate layers, add 200 μL 10 mg/mL BHT in ethyl acetate, evaporate to dryness under vacuum at 30-35° (beware of bumping). Rinse out flask with three 3 mL portions of dichloromethane, add rinses to the SPE cartridge, wash with 5 mL dichloromethane, elute with 5 mL dichloromethane:MeOH 75:25. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, vortex, filter (0.2 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 Brownlee RP-18 Spheri-10 MPLC
Column: 250 × 4.6 5 μm C18 (Alltech)
Mobile phase: MeOH:buffer 53:47 (Buffer was 1.15 g $(NH_4)H_2PO_4$ in 950 mL water, adjust pH to 7.0 with dilute ammonia, make up to 1 L with water.)
Flow rate: 1
Injection volume: 50
Detector: UV 298

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Extracted: oxfendazole, mebendazole, metabolites
Simultaneous: chloramphenicol
Noninterfering: amprolium, chlortetracycline, erythromycin, levamisole, morantel, oxytetracycline, phenothiazine, sulfadimethoxine, sulfamethazine, sulfaquinoxaline

---

KEY WORDS
cow; liver; SPE

REFERENCE
LeVan,L.W.; Barnes,C.J. Liquid chromatographic method for multiresidue determination of benzimidazoles in beef liver and muscle: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 487–493.

SAMPLE
Matrix: vegetables
Sample preparation: Condition a 10 mL 500 mg Bond Elut LRC, PRS (propylsulfonic acid) SPE cartridge with 10 mL conditioning solution, 3 mL MeOH, and 5 mL ethyl acetate. Homogenize with an equal amount of water. 10 g Potato homogenate or 20 g sweet potato homogenate + 20 mL ethyl acetate, shake on a reciprocating shaker for 10 min, centrifuge at 2500-3000 rpm for 15 min, repeat the extraction, add the ethyl acetate layers to the SPE cartridge, wash with 5 mL ethyl acetate, dry the SPE cartridge under vacuum, elute with 9.8 (potato) or 4.8 (sweet potato) mL elution solution, make up to 5 (sweet potato) or 10 (potato) mL with elution solution, dilute with mobile phase if necessary, inject a 50 μL aliquot. (Prepare conditioning solution by mixing 40 mL water and 2 mL 85% phosphoric acid, make up to 200 mL with MeOH. Prepare elution solution by dissolving 2.8 g KH_2PO_4 in 100 mL water, add 60 mL MeCN, make up to 200 mL water.)

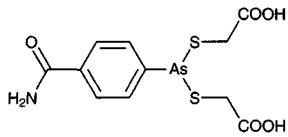
HPLC VARIABLES
Column: 125 × 4.6 5 μm PartiSphere SCX (benzenesulfonic acid) (Whatman)
Mobile phase: MeCN:buffer 25:75, adjusted to pH 3.4 with 85% phosphoric acid. (Prepare mobile phase by dissolving 13.6 g KH_2PO_4 in 1 L water, add 500 mL MeCN, make up to 2 L with water, adjust pH to 3.4 with 85% phosphoric acid.)
Column temperature: 25
Flow rate: 1
Injection volume: 50
Detector: F ex 305 em 380

CHROMATOGRAM
Retention time: 5
Limit of detection: 2.5 ppb
Limit of quantitation: 5 ppb

KEY WORDS
potato; sweet potato; SPE

REFERENCE
Arenas,R.V.; Rahman,H.; Johnson,N.A. Determination of thiabendazole residues in white and sweet potatoes by liquid chromatography with fluorescence detection, *J.AOAC Int.*, **1995**, *78*, 1455–1458.

Thiacetarsamide



Molecular formula: $C_{11}H_{12}AsNO_5S_2$
Molecular weight: 377.27
CAS Registry No.: 531-72-6, 7681-85-8 (di Na salt)
Merck Index: 831

SAMPLE
Matrix: solutions

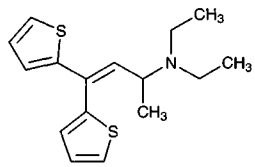
HPLC VARIABLES
Column: 250 × 4.6 6 μm Zorbax C-8
Mobile phase: MeOH:0.25 mM pH 7 sodium phosphate buffer containing 0.125 mM EDTA
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 1 (thiacetarsamide), 4.5 (p-arsenobenzamide, the major degradation product)

KEY WORDS
protect from light

REFERENCE
Leadbetter,M.G.; Allen,E.H. Liquid chromatographic determination of the composition of thiacetarsamide solution, *J.Liq.Chromatogr.*, **1986**, *9*, 1075–1094.

Thiambutene



Molecular formula: $C_{16}H_{21}NS_2$
Molecular weight: 291.48
CAS Registry No.: 96-14-6
Merck Index: 9429

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

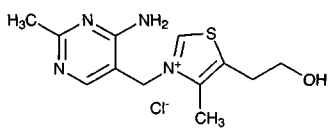
HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Thiamine



Molecular formula: $C_{12}H_{17}ClN_4OS$
Molecular weight: 300.81
CAS Registry No.: 59-43-8, 67-03-8 (HCl), 532-43-4 (mononitrate)
Merck Index: 9430

SAMPLE
Matrix: blood
Sample preparation: 1 mL Hemolysate + 30 µL 4 µM IS in 100mM HCl, shake thoroughly. Slowly add 2 mL MeOH, mix, let stand for 30 min. Centrifuge at 2000 g for 10 min. Add 50 µL freshly prepared 30.4 mM potassium ferricyanide and 50 µL 0.8 mM NaOH to 1 mL supernatant. Filter (0.45 µm) and inject a 50 µL aliquot.